American Journal of Hematology 53:66–71 (1996)

Activation of a Recombinant Human Factor VII Structural Analogue Alters Its Affinity of Binding to Tissue Factor

Sampath Sridhara, Shu Chaing, Katherine A. High, Morris A. Blajchman, and Bryan J. Clarke

Departments of Pathology (S.S., M.A.B., B.J.C.) and Biomedical Sciences (B.J.C.), McMaster University, Hamilton, Ontario; Blood Transfusion Services (M.A.B.), Canadian Red Cross Society, Hamilton, Ontario, Canada; Hematology Division, Children's Hospital of Philadelphia (K.A.H.), Philadelphia, Pennsylvania; Department of Medicine and Pathology, University of North Carolina at Chapel Hill (S.C.), Chapel Hill, North Carolina

A competitive enzyme-linked immunoadsorbent assay (ELISA) technique has been developed to facilitate quantitative analysis of the earliest step in the initiation of the extrinsic pathway of coagulation, i.e., complex formation of factor VII/VIIa with tissue factor. The ELISA measures the binding of biotinylated human plasma factor VII to relipidated recombinant human tissue factor. Quantitation of the relative affinity (expressed as IC_{50}) of any factor VII molecular population or structural analogue for tissue factor can be determined by competitive binding. Subnanomolar concentrations of both wild-type recombinant human factor VII (rFVII) and rFVII(R152Q), a mutation at the FVII activation site, competed effectively with biotinylated plasma-derived factor VII in binding to tissue factor. In contrast, the affinity of rFVII(R79Q), a mutation in the first epidermal growth factor-like domain, was 12-fold lower. Following activation of rFVII(R79Q), its affinity for tissue factor and enzymatic activity increased 4-fold and 6-fold, respectively. For wild-type rFVII, enzymatic activity rose significantly following activation. However, its affinity for tissue factor was unchanged. We conclude that both the activation state of factor VII and the mutation of amino-acid residues within the first epidermal growth factor-like domain may alter the affinity of factor VII for tissue factor. © 1996 Wiley-Liss, Inc.

Key words: factor VII, tissue factor, epidermal growth factor domain

INTRODUCTION

Coagulation factor VII (FVII) is a 50-kDa, multidomain, vitamin K-dependent glycoprotein that is synthesized in the liver and secreted into the bloodstream as an inactive zymogen precursor of the serine protease factor VIIa (FVIIa). After binding to its specific cell-surface receptor tissue factor in the presence of calcium, single-chain zymogen FVII is converted to two-chain enzymatically-active FVIIa by cleavage of the Arg_{152}-Ile_{153} peptide bond. The FVIIa-tissue factor complex can then rapidly activate its principal substrates, factor IX and factor X, by limited proteolysis, leading to thrombin formation and ultimately a fibrin clot [1–4].

We have previously shown that a monoclonal antibody specific for the first epidermal growth factor-like (EGF-1) domain of human FVII blocked both the binding of FVII to tissue factor and its activation to FVIIa, suggesting that the EGF-1 domain of FVII may be essential for complex formation with tissue factor [5]. Recently, we confirmed the above finding by demonstrating that a naturally-occurring R79Q mutation within the EGF-1 domain of FVII markedly decreased FVII binding to human tissue factor [6,7]. As an extension of this work, plasma-derived human FVII (pdFVII) was biotinylated and utilized in a competitive enzyme-linked immunoadsorbent assay (ELISA) to quantitate the competition between structural mutants of recombinant FVII and biotinylated pdFVII in binding to tissue factor. Recombinant, wild-type human FVII and two FVII structural analogues, R79Q and R152Q, were employed in the present study. This competitive

Received for publication June 2, 1995; accepted April 10, 1996.

Address reprint requests to Dr. Bryan J. Clarke, McMaster University Medical Centre, Department of Pathology, HSC-4N65, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada.

© 1996 Wiley-Liss, Inc.

ELISA allows rapid analysis of the relative affinity of nanogram quantities of either purified or unpurified FVII molecular populations for tissue factor. A preliminary report of this work has been published in abstract form [8].

MATERIALS AND METHODS

Materials

Purified pdFVII was purchased from Enzyme Research Laboratories, Inc. (South Bend, IN). Recombinant tissue factor (TF) apoprotein, expressed in *Escherichia coli* and purified to homogeneity, was a generous gift of Dr. R. Kelley (Genentech, Inc., South San Francisco, CA). Recombinant FVII proteins - wild-type, (R79Q) and (R152Q) were permanently expressed in the human kidney cell line 293 using the expression vector pCMV5 and purified to homogeneity using adsorption on Q-Sepharose followed by affinity chromatography on a calcium-dependent anti-FVII monoclonal antibody sepharose column, as previously described [7]. Affinity-purified goat anti-rabbit IgG (heavy and light chain) and alkaline phosphatase-conjugated streptavidin were purchased from Jackson Immuno Research Laboratories (BioCan Scientific, Mississauga, Ontario, Canada). Monospecific polyclonal rabbit anti-human FVII sera were obtained from Diagnostica Stago (Wellmark Diagnostics, Guelph, Ontario, Canada). Monoclonal antibody 231-7 to human FVII was produced and purified in our laboratory [9]. Factor X was purified from normal pooled human plasma as previously described [10]. Factor Xa was prepared by Russells viper venom activation of factor X and purified by DEAE-Sephadex A50 chromatography [11]. Bovine serum albumin, fraction V, was from Gibco Laboratories (Gibco Canada, Inc., Burlington, Ontario, Canada). Normal pooled human plasma was prepared by pooling citrated plasma from 20 healthy donors, and was stored in aliquots at −70°C. Tween-20 was from Pierce Chemical Company (Chromatographic Specialties Inc., Brockville, Ontario, Canada). N-hydroxysuccinimidobiotin (NHS-Biotin) was obtained as a 75-mM stock solution in dimethylsulfoxide from Bio-Rad Laboratories (Mississauga, Ontario, Canada). Benzamidine HCl and dansyl-Glu-Gly-Arg chloromethyl ketone (dansyl-Glu-Gly-Arg-CH_2Cl) were obtained from Calbiochem (San Diego, CA). L-α-phosphatidylcholine, type VE (egg yolk), L-α-phosphatidyl-L-serine (bovine brain), poly-L-lysine HBr (Molecular weight 100,000), n-octyl-β-D-glycopyranoside, disodium-p-nitrophenyl phosphate (PNPP), nitro blue tetrazolium (NBT), and bromochloroindolyl phosphate (BCIP) were purchased from Sigma Chemical Company (St. Louis, MO). Platelin (rabbit brain phospholipids) was obtained from Organon Teknika, Canada (Scarborough, Ontario, Canada). S-2222(N-benzoyl-L-isoleucyl-L-glutamyl-glycyl-L-arginine-p-nitroanilide), a chromogenic peptide substrate for factor Xa, was obtained from Kabi Diagnostica (Helena Laboratories, Mississauga, Ontario, Canada). ELISA assays were performed in flat-bottomed 96-well Immulon II microtiter plates (Fisher Scientific, Toronto, Ontario, Canada).

Factor VII Antigen Concentration

FVII antigen concentration was determined by ELISA using the factor VII-specific monoclonal antibody 231-7 [9] as the trapping antibody, monospecific polyclonal rabbit anti-human FVII sera as primary antibody, and alkaline phosphatase-conjugated goat anti-rabbit IgG as secondary antibody [12]. Normal pooled plasma was used as a standard and assumed to have a FVII antigen concentration of 450 ng/ml [13].

Clotting and Amidolytic Activity Measurements

Clotting activity of the various FVII samples was measured by prothrombin time (PT) assay using the Fibrintimer CoaSYSTEM analyzer (Labor GmbH, Hamburg, Germany) as previously described [6]. Briefly, FVII test samples and normal pooled plasma were diluted in 0.05 M imidazole-HCl, pH 7.4, containing 0.1 M NaCl and 3.5 mg/ml bovine serum albumin (imidazole buffer). A 100-μl volume of diluted test sample or normal pooled plasma was gently mixed with 100 μl of FVII-depleted plasma and incubated at 37°C. Twenty ng of relipidated recombinant TF [6] in 200 μl of 0.15 M NaCl supplemented with 3.5 mg/ml bovine serum albumin, and 32.5 mM $CaCl_2$, were added to initiate coagulation, and the PT was noted. A standard log-log plot of PT vs. clotting activity was constructed using various dilutions of normal pooled plasma, and the clotting activity of test samples was determined by reference to this graph. FVII in normal pooled plasma has a specific clotting activity of 2,200 U/mg FVII antigen [13]. FVII activation was also measured by amidolytic assay using S-2222 chromogenic substrate [14].

Biotinylation of Human Plasma Factor VII

Human pdFVII was biotinylated as described by Savage et al. [15]. Briefly, the pdFVII was dialyzed against 0.05 M carbonate-bicarbonate buffer, pH 8.5 (4 × 250 ml), overnight at 4°C. A working stock solution (1.24 mM) of NHS-biotin was prepared in dimethylsulfoxide. To 120 μl of a sample containing 15 μg pdFVII, 4.8 nmol of NHS-biotin were added, mixed gently, and incubated in an ice bath for 2 hr. The molar ratio of NHS-biotin to FVII was thus 16:1. After 2 hr, 10 μl of 1 M Tris-HCl, pH 8.0, were added to the reaction mixture to inactivate any excess NHS-biotin, followed by further incubation for 15 min in an ice bath. Then 10 μl of bovine serum albumin (50 mg/ml in H_2O) were added as a carrier protein, and the sample was dialyzed against 0.05 M Tris-buffered saline, pH 7.5, overnight at 4°C (4 × 250 ml).

After dialysis, biotinylated pdFVII was divided into 20-μl aliquots and stored at −70°C.

Competitive ELISA of Biotinylated pdFVII Binding to Immobilized Recombinant Tissue Factor

Recombinant tissue-factor apoprotein was relipidated into phosphatidylcholine and phosphatidylserine lipid vesicles (60:40 by weight) as described elsewhere [6]. Relipidated recombinant TF (11.5 ng) was coated onto flat-bottom Immulon II microtiter plates in 100 μl of carbonate antigen coating buffer overnight at 4°C. Pure phospholipid vesicle-coated wells (without TF) were used as controls. After washing the plates three times in 0.15 M NaCl, nonspecific binding sites on the plates were blocked with 3.5-mg/ml bovine serum albumin in TBS-T buffer (10 mM Tris, pH 8.0, 0.15 M NaCl, 0.025% Tween-20, 10 mM $CaCl_2$) for 2 hr at room temperature. After washing three times in TBS-T, 100 μl of biotinylated pdFVII in TBS-T + bovine serum albumin buffer were added in triplicate to recombinant TF-coated wells and incubated for 2 hr at room temperature. To generate a standard curve, biotinylated pdFVII in the concentration range of 1–16 ng/ml was added in the absence of any inhibitors. For competitive ELISA, a fixed amount of biotinylated pdFVII (5 ng/ml final concentration) was gently mixed with varying concentrations of the inhibitors, i.e., wild-type rFVII, rFVII (R152Q), and rFVII (R79Q), and added to the wells. The molar ratio of inhibitors to biotinylated pdFVII varied from 0.5:1–10:1. After washing four times in TBS-T buffer, biotinylated pdFVII bound to TF was detected by adding 100 μl of 0.75-μg/ml streptavidin conjugated to alkaline phosphatase in TBS-T buffer + bovine serum albumin, followed by incubation for 1 hr at room temperature. After washing four times, 100 μl of the chromogenic substrate p-nitrophenylphosphate in diethanolamine buffer (1 mg/ml) were added, followed by incubation for 1 hr at room temperature. Color development was stopped after 1 hr by adding 25 μl of 1 M NaOH, and sample absorbance was read at 405 nm in a microplate autoreader (Model EL309, BioTek Instruments, Burlington, VT). After subtraction of background nonspecific binding (routinely <0.1 OD units), semilogarithmic plots of percent biotinylated pdFVII bound vs. log of the inhibitor concentration was fitted via linear regression analysis, and the IC_{50} (inhibitor concentration needed to achieve 50% reduction of binding of biotinylated pdFVII to TF) value for each rFVII structural analogue was derived from the respective graph [16]. The decrease in biotinylated pdFVII binding to TF was thus a function of both the affinity and molar concentration of the rFVII inhibitor.

Activation of rFVII Molecules

Both wild-type rFVII and rFVII(R79Q) were activated to FVIIa in the absence of tissue factor, using purified factor Xa. The presence of Ca^{2+}, phospholipids (platelin), and a positively-charged surface such as poly-L-lysine [17] was required for maximum cleavage of FVII. Activation was carried out in 10 mM Tris-buffered saline, pH 8.0, containing final concentrations of 5 mM Ca^{2+}, 3.5 mg/ml bovine serum albumin, and 0.025% Tween-20. Briefly, 5 μl of rFVII (25 μg/ml) were gently mixed in a microfuge tube with 5 μl of phospholipids, 5 μl of purified factor Xa (0.5 μg/ml), and 2 μl of poly-L-lysine (5μg/ml), and the final reaction volume was adjusted to 20 μl with buffer. The samples were incubated at 37°C for 90 min. The weight ratio of enzyme to FVII in the reaction was thus 1:50, and that of poly-L-lysine to FVII was 1:12.5. Control reactions containing only rFVII plus buffer, or rFVII, buffer, and phospholipids, were performed simultaneously. Following activation, the reaction was stopped by adding 10 μl of an inhibitor containing 75 mM benzamidine and 0.3 mM dansyl-glu-gly-arg CH_2Cl in H_2O, for both ELISA binding assays and IC_{50} determination. For clotting-activity measurements, the reaction was stopped by diluting the samples in calcium-free imidazole buffer and storing on ice. Residual factor Xa activity in the test samples did not have any significant effect on the PT assay. The completeness of factor Xa digestion of rFVII molecules was determined using reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis [18].

RESULTS

Functional Activity of Biotinylated pdFVII

In the present work we initially determined if biotinylation, rather than iodination, might serve as a useful labelling procedure for the quantitative analysis of the binding characteristics of purified pdFVII to solid-phase recombinant TF. Biotinylated pdFVII was therefore analyzed by SDS-PAGE, S-2222 amidolytic assay, PT assay, and binding to recombinant TF-coated microtiter wells. Relative to native pdFVII, biotinylated pdFVII had mean amidolytic and clotting activities of 88% and 61%, respectively. Biotinylated pdFVII was chemically intact, as indicated by its migration as a single 50-kDa band on SDS-PAGE analysis (data not shown). Figure 1 shows the time course for binding to solid-phase recombinant TF of equivalent concentrations (10 ng/ml or 0.1 nM) of native and biotinylated pdFVII. FVII binding was quantitated by ELISA, using rabbit anti-human factor VII as primary antibody and alkaline phosphatase-conjugated goat anti-rabbit IgG as secondary antibody. After 2 hr of incubation at room temperature, the relative binding of biotinylated pdFVII to solid-phase recombinant TF compared favorably to native pdFVII (Fig. 1). Further experiments indicated that the binding of biotinylated pdFVII to recombinant TF-coated microtiter wells, as detected by chromogenic assay using

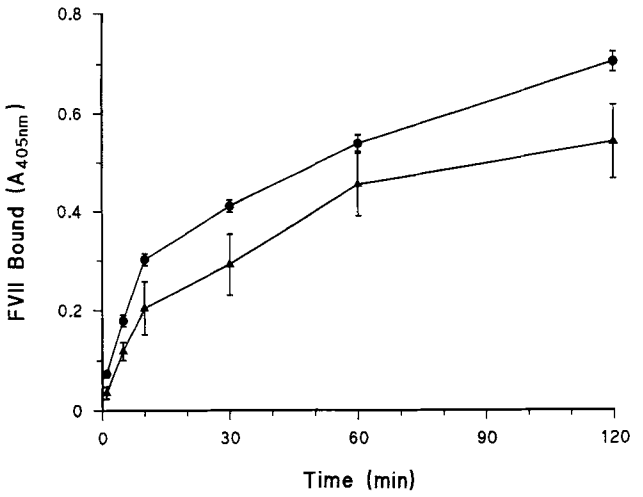

Fig. 1. Time course of binding of native (•) and biotinylated (▲) pd FVII at 10 ng/ml concentration (0.2 nM) to immobilized recombinant tissue factor, as measured by ELISA. Data are means ± SEM.

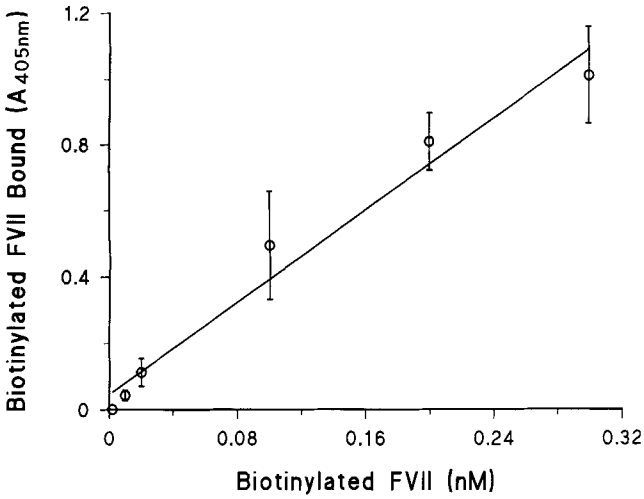

Fig. 2. Dose-response of binding of biotinylated pdFVII to immobilized recombinant tissue factor, as measured by ELISA. Data are means ± SEM.

streptavidin-alkaline phosphatase and PNPP, was saturable at 0.4 nM biotinylated pdFVII (data not shown) and linear ($r = 0.98$) in the concentration range 0.02–0.32 nM (Fig. 2). We concluded that biotinylation of purified pdFVII was an efficient approach to the evaluation of FVII binding to solid-phase recombinant TF.

Competitive Inhibition of Biotinylated pdFVII Binding to Recombinant TF: Effect of Unactivated rFVII Analogues

We recently described a patient with homozygous mutations in both the EGF-1 domain, R79Q, and the factor Xa cleavage site, R152Q, of FVII [7]. In the process of analyzing the effects of the two mutations on the function of FVII, the recombinant proteins wild-type rFVII, rFVII(R152Q), and rFVII(R79Q) were separately expressed in and purified to homogeneity from human 293 cells [7]. Since the binding of biotinylated pdFVII to solid-phase recombinant TF was linearly related to FVII concentration, we utilized this assay system to quantitatively assess the competitive binding characteristics of rFVII analogues as IC_{50} values. When a fixed concentration of biotinylated pdFVII (0.1 nM, i.e., 5 ng/ml) was incubated with increasing molar concentrations of competing rFVII, both unactivated rFVII wild-type and rFVII (R152Q) were equally effective as competitive inhibitors. At a 1.5-fold molar excess of these inhibitors, the binding of biotinylated pdFVII was decreased by 50% (Fig. 3 and Table I). In contrast, unactivated rFVII(R79Q) was a very weak competitive inhibitor, exhibiting an affinity more than an order of magnitude lower than that of unactivated wild-type rFVII (Fig. 3 and Table I).

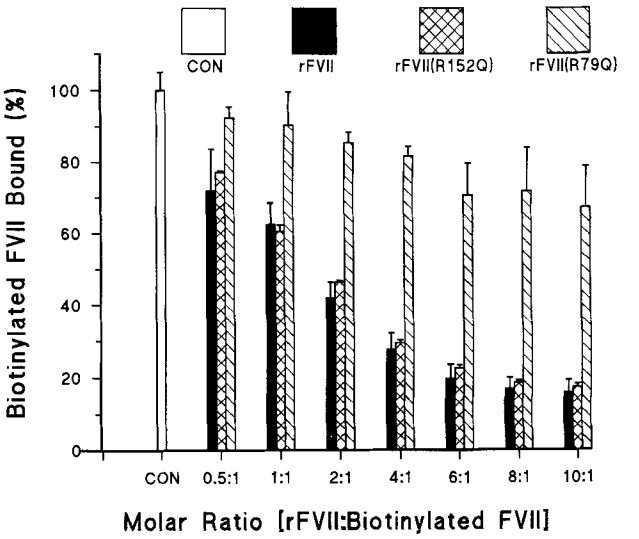

Fig. 3. Competitive inhibition of binding of biotinylated pdFVII to immobilized recombinant tissue factor by rFVII structural analogues. Con, control value (100%) in absence of inhibitor. rFVII, rFVII(R152Q), and rFVII(R79Q) represent various recombinant FVII molecules, as described in the text. Data are means ± SEM.

TABLE I. Effect of Proteolytic Activation of rFVII Analogues on Binding to Tissue Factor

	IC_{50} (nM)[a]	
Sample	rFVII (not activated)	rFVII activated by factor Xa
rFVII	0.16 ± 0.08	0.15 ± 0.04
rFVII(R79Q)	1.94 ± 0.54	0.56 ± 0.10
rFVII(R152Q)	0.15 ± 0.04	

[a] IC_{50} were measured by competitive inhibition of biotinylated pdFVII binding to recombinant TF. Data are means ± SEM.

TABLE II. Effect of Proteolytic Activation of rFVII Analogues on Clotting Activity

Treatment	Clotting activity (units/mg protein)[a]	
	rFVII	rFVII(R79Q)
Buffer only	1,700 ± 260	440 ± 60
Buffer + phospholipid	1,720 ± 120	500 ± 20
Buffer + phospholipid + factor Xa	4,540 ± 760	3,080 ± 420

[a]Clotting activities shown represent mean ± SEM with reference to normal pooled plasma. The rFVII(R152Q) analogue had no clotting activity (data not shown). After activation, both unactivated and activated samples were diluted to a FVII antigen concentration of 500 ng/ml, and clotting activities were determined.

Competitive Inhibition of Biotinylated pdFVII Binding to Recombinant TF: Effect of Proteolytic Activation of rFVII Analogues

Both wild type rFVII and rFVII(R79Q) were activated to rFVIIa (in the absence of tissue factor) with purified factor Xa protease in the presence of poly-L-lysine, phospholipids, and Ca^{2+} [17]. Analysis of the rFVII molecules before and after enzyme digestion was performed by reducing SDS-PAGE and Western blots using polyclonal rabbit anti-human FVII-specific antisera [7]. The cleavage of both wild-type rFVII and rFVII(R79Q) to rFVIIa was essentially complete (data not shown). The IC_{50} for both unactivated and activated rFVII molecules was determined. As shown in Table I, the IC_{50} for activated rFVII(R79Q) decreased approximately 4-fold from 1.94 nM to 0.56 nM, whereas the IC_{50} for unactivated wild-type rFVII, wild-type rFVIIa, and rFVII(R152Q) were essentially identical at 0.16 nM, 0.15 nM, and 0.15 nM, respectively. Thus, the proteolytic activation of wild-type rFVII had little effect on its affinity for immobilized recombinant TF, whereas activation of the rFVII(R79Q) analogue markedly increased its affinity for solid-phase recombinant TF.

Clotting Activity of rFVII Analogues

To confirm the functional relevance of the observed IC_{50} values, the clotting activities of unactivated and activated rFVII molecules were measured using the PT assay (Table II). At physiological concentrations, unactivated rFVII(R79Q) had only 25% of the clotting activity of wild-type rFVII, a result consistent with its poor affinity for TF. After activation by factor Xa, rFVII(R79Q) increased in absolute clotting activity by 6-fold (Table II) and had 68% of the clotting activity of activated wild-type rFVII.

DISCUSSION

An ELISA for quantitation of the binding of biotinylated pdFVII to relipidated immobilized recombinant TF has been described. Direct competition between structural mutants of rFVII and biotinylated pdFVII in binding to immobilized recombinant TF could thus be demonstrated and expressed as the relative affinity constant, IC_{50}. Although radioiodination has been widely used for labelling coagulation FVII [19–23], the advantage of biotinylation lies in its simplicity and safety, and in the chemical stability of biotinylated proteins. The biotinylation of pdFVII did not adversely affect either its enzymatic activity or its ability to bind to immobilized recombinant TF. The time course of binding of biotinylated pdFVII to immobilized recombinant TF was similar to that of native pdFVII. Based on these data, we concluded that the biotinylation of pdFVII was a satisfactory labelling procedure, enabling us to pursue further quantitative analyses of the interaction of FVII and TF.

In competitive ELISA experiments, unactivated wild-type rFVII and rFVII(R152Q) effectively competed with biotinylated pdFVII for binding to immobilized recombinant TF. In contrast, the IC_{50} for unactivated rFVII(R79Q) was more than an order of magnitude lower than those observed for the other rFVII-TF interactions (Table I). This data is supported by recent TF binding measurements, which described a 7.5-fold lower affinity for unactivated rFVII(R79Q) as compared to unactivated wild-type rFVII [24]. The clotting activity of unactivated rFVII (R79Q), as measured by PT assay (Table II), was about 25% of that of wild-type rFVII. In agreement with the latter data, Takamiya et al. [25] have described 2 additional patients with mutations at amino-acid residue 79 in the EGF-1 domain of FVII. In the first case, the subject had a homozygous R79Q substitution, while in the second case, the subject was found to be heterozygous for an R79W substitution. Both of these FVII variants of arginine-79 were found to exhibit markedly reduced FVII clotting activity [25]. Thus, the competitive ELISA binding experiments described herein, clotting ability measurements in three distinct kindreds [7,25], and recent molecular modelling studies of the FVIIa-TF complex [26] are all consistent with the interpretation that mutations at the arginine-79 position of FVII result in impaired interaction with TF.

An interesting and novel observation was the finding that the activation of rFVII(R79Q) to rFVIIa(R79Q) resulted in enhanced binding to recombinant TF and increased clotting activity relative to unactivated

rFVII(R79Q) (Table II). The marked increase in functional activities of rFVIIa(R79Q) may explain the tissue factor-dependent activity of rFVII(R79Q) observed by Kazama et al. [27]. In contrast, the activation of wild-type rFVII to rFVIIa did not significantly affect its affinity for recombinant TF but, as expected, was associated with an increase in observed clotting activity. These data support the important concept that FVII activation causes a conformational change in the molecule [28], allowing expression of neotissue-factor-binding epitope(s) in the heavy chain of FVIIa [29–31].

ACKNOWLEDGMENTS

The authors thank Dr. R. Kelley, Genentech, Inc., for generously providing recombinant human TF apoprotein for use in these studies. The authors also gratefully acknowledge the Heart and Stroke Foundation of Ontario (B.J.C.), the Canadian Red Cross Society (M.A.B.), and the National Institutes of Health, Bethesda, Maryland (K.A.H.) for supporting this work.

REFERENCES

1. Hagen FS, Gray CL, O'Hara P, Grant F, Saari GC, Woodbury RG, Hart CE, Inslay M, Kisiel W, Kurachi K, Davie EW: Characterization of a cDNA coding for human factor VII. Proc Natl Acad Sci USA 83:2412–2416, 1986.
2. Bach RR: Initiation of coagulation by tissue factor. CRC Crit Rev Biochem 23:339–368, 1988.
3. Nemerson Y: Tissue factor and hemostasis. Blood 71:1–8, 1988.
4. Bom VJ, Bertina RM: The contributions of Ca^{2+}, phospholipids and tissue-factor apoprotein to the activation of human blood coagulation factor X by activated factor VII. Biochem J 265:327–336, 1990.
5. Clarke BJ, Ofosu FA, Sridhara S, Bona RD, Rickles FR, Blajchman MA: The first epidermal growth factor domain of human coagulation factor VII is essential for binding with tissue factor. FEBS Lett 298:206–210, 1992.
6. Sridhara S, Clarke BJ, Ofosu FA, High KA, Blajchman MA: The direct binding of human factor VII in plasma to recombinant human tissue factor. Thromb Res 70:307–316, 1993.
7. Chaing S, Clarke B, Sridhara S, Chu K, Friedman P, Van Dusen W, Roberts HR, Blajchman M, Monroe DM, High K: Severe factor VII deficiency due to mutations abolishing the cleavage site for activation and altering binding to tissue factor. Blood 83:3524–3535, 1994.
8. Sridhara S, Clarke BJ, High KA, Blajchman MA: The effect of recombinant human factor VII structural analogues on the binding of biotinylated plasma factor VII to recombinant human tissue factor. Thromb Haemost 69:613, 1993.
9. Cerskus AL, Ofosu FA, Birchall KL, Clarke BJ, Modi GJ, Johnston M, Blajchman MA: The immunodepletion of factor VII from human plasma using a monoclonal antibody. Br J Haematol 61:467–475, 1985.
10. Modi GJ, Blajchman MA, Ofosu FA: The isolation of prothrombin, factor IX and factor X from human factor IX concentrate. Thromb Res 36:537–547, 1984.
11. Ofosu FA, Modi GJ, Cerskus AL, Blajchman MA, Hirsh J: Activation of factor X and prothrombin in antithrombin III depleted plasma. Thromb Res 23:331–345, 1981.
12. Mills JK, Ofosu FA, Safa TK, Siegel RS, Lessin LS: Plasma factor VII and thrombin-antithrombin III levels indicate increased tissue factor activity in sickle cell patients. Br J Haematol 81:539–544, 1992.
13. Howard PR, Bovill EG, Pike J, Church WR, Tracy RP: Factor VII antigen levels in a healthy blood donor population. Thromb Haemost 72:21–27, 1994.
14. Seligsohn U, Osterud B, Rappaport S: Coupled amidolytic assay for factor VII: Its use with a clotting assay to determine the activity state of factor VII. Blood 52:978–988, 1978.
15. Savage D, Mattson G, Desai S, Nielander G, Morgensen S, Conklin E, eds.: Biotinylation reagents. In "Avidin-Biotin Chemistry: A Handbook." Rockford, IL: Pierce Chemical Co., 1992, pp 25–88.
16. Bylund DB, Yamamura HI: Methods for receptor binding. In Yamamura HI, (eds): "Methods in Neurotransmitter Receptor Analysis." New York: Raven Press, Ltd. 1990, pp 1–35.
17. Pedersen AH, Lund-Hansen T, Bisgaard-Frantzen H, Olsen F, Petersen LC: Auto-activation of human recombinant coagulation factor VII. Biochemistry 28:9331–9336, 1989.
18. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354, 1979.
19. Sakai T, Lund-Hansen T, Thim L, Kisiel W: The γ-carboxyglutamic acid domain of human factor VIIa is essential for its interaction with cell surface tissue factor. J Biol Chem 265:1890–1894, 1990.
20. Wildgoose P, Kazim AL, Kisiel W: The importance of residues 195–206 of human blood clotting factor VII in the interaction of factor VII with tissue factor. Proc Natl Acad Sci USA 87:7290–7294, 1990.
21. Wildgoose P, Berkner KL, Kisiel W: Synthesis, purification, and characterization of an Arg152Glu site-directed mutant of recombinant human blood clotting factor VII. Biochemistry 29:3413–3420, 1990.
22. Toomey JR, Smith KJ, Stafford DW: Localization of the human tissue factor recognition determinant of human factor VIIa. J Biol Chem 266:19198–19202, 1991.
23. Ruf W, Kalnik MW, Lund-Hansen T, Edgington TS: Characterization of factor VII association with tissue factor in solution. J Biol Chem 266:15719–15725, 1991.
24. O'Brien DP, Kemball-Cook G, Hutchinson AM, Martin DMA, Johnson DJD, Byfield PGH, Takamiya O, Tuddenham EGD, McVey JH: Surface plasmon resonance studies of the interaction between factor VII and tissue factor. Demonstration of defective tissue factor binding in a variant FVII molecule (FVII-R79Q). Biochemistry 33:14162–14169, 1994.
25. Takamiya O, Kemball-Cook G, Martin DMA, Cooper DM, Von Felten A, Meili E, Hann I, Prangnell DR, Lumley H, Tuddenham EGD, McVey JH: Detection of missense mutations by single-strand conformational polymorphism (SSCP) analysis in five dysfunctional variants of coagulation factor VII. Hum Mol Genet 2:1355–1359, 1993.
26. Banner D, D'Avey A, Chene C, Winkler F, Suha A, Konigsberg W, Nemerson Y, Kirchhofer D: The crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor. Nature 380:41–46, 1996.
27. Kazama Y, Foster DC, Kisiel W: Evidence that an Arg79-Gln substitution in human factor VII is not associated with a reduction in coagulation activity. Blood Coag Fibrinol 3:697–702, 1992.
28. Higashi S, Nishimura H, Aita K, Iwanaga S: Identification of regions of bovine factor VII essential for binding to tissue factor. J Biol Chem 269:18891–18898, 1994.
29. O'Brien D, Gale K, Anderson J, McVey J, Miller G, Meade T, Tuddenham E: Purification and characterization of factor VII 304-Gln: A variant molecule with reduced activity isolated from a clinically unaffected male. Blood 78:132–140, 1991.
30. Wildgoose P, Foster D, Schiodt J, Wilberg FC, Birktoft J, Petersen LC: Identification of a calcium binding site in the protease domain of human blood coagulation factor VII: Evidence for its role in factor VII-tissue factor interaction. Biochemistry 32:114–119, 1993.
31. Matsushita T, Kojima T, Emi N, Takahashi I, Saito H: Impaired human tissue factor-mediated activity in blood clotting factor VII_{Nagoya} ($Arg^{304}\rightarrow Trp$). J Biol Chem 269:7355–7363, 1994.